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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (c).

INVENTOR(S)					
Given Name (first and middle [if any])		Family Name or Surname		Residence (City and either State or Foreign Country)	
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<input checked="" type="checkbox"/> Additional inventors are being named on the <u>1</u> separately numbered sheets attached hereto					
TITLE OF THE INVENTION (280 characters max)					
Therapeutic Uses for Aminosterol Compounds					
CORRESPONDENCE ADDRESS					
Direct all correspondence to:					
<input type="checkbox"/> Customer Number		<input type="text"/>		Place Customer Number Bar Code Label here	
OR Type Customer Number here					
<input checked="" type="checkbox"/> Firm or Individual Name		Magainin Pharmaceuticals Inc.			
Address		5110 Campus Drive			
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City		Plymouth Meeting	State	Pennsylvania	ZIP 19462
Country		U.S.A.	Telephone	610-941-4020	Fax 610-941-5399
ENCLOSED APPLICATION PARTS (check all that apply)					
<input checked="" type="checkbox"/> Specification Number of Pages		27		<input checked="" type="checkbox"/> Small Entity Statement	
<input checked="" type="checkbox"/> Drawing(s) Number of Sheets		15		<input type="checkbox"/> Other (specify) <input type="text"/>	
METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT (check one)					
<input checked="" type="checkbox"/> A check or money order is enclosed to cover the filing fees				FILING FEE AMOUNT (\$)	
<input type="checkbox"/> The Commissioner is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number <input type="text"/>				75.00	
The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.					
<input checked="" type="checkbox"/> No					
<input type="checkbox"/> Yes, the name of the U.S. Government agency and the Government contract number are _____					

Respectfully submitted,

Date

7/13/00

SIGNATURE

W. Lee Maloy

REGISTRATION NO.

42,834

TYPED or PRINTED NAME

610-941-5242

(if appropriate)

Docket Number:

5032M

TELEPHONE

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is used by the public to file (and by the PTO to process) a provisional application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the complete provisional application to the PTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, Washington, D.C., 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Box Provisional Application, Assistant Commissioner for Patents, Washington, D.C., 20231.

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PROVISIONAL APPLICATION COVER SHEET
Additional Page



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Docket Number		5032M	Type a plus sign (+) inside this box →	+
INVENTOR(S)/APPLICANT(S)				
Given Name (first and middle [if any])	Family or Surname	Residence (City and either State or Foreign Country)		
Mike	McLane	Lansdale, PA		

Number 2 of 2



**STATEMENT CLAIMING SMALL ENTITY STATUS
(37 CFR 1.9(f) & 1.27(c))--SMALL BUSINESS CONCERN**

Docket Number (Optional)
5032M

Applicant, Patentee, or Identifier: Maqainin Pharmaceuticals Inc.
Application or Patent No.: Provisional Patent Application
Filed or Issued: 7/13/00
Title: Therapeutic Uses for Aminosterol Compounds

I hereby state that I am
☐ the owner of the small business concern identified below.
☒ an official of the small business concern empowered to act on behalf of the concern identified below:

NAME OF SMALL BUSINESS CONCERN Maqainin Pharmaceuticals Inc.

ADDRESS OF SMALL BUSINESS CONCERN 5110 Campus Drive, Plymouth Meeting, Pennsylvania,

I hereby state that the above identified small business concern qualifies as a small business concern as defined in 13 CFR Part 121 for purposes of paying reduced fees to the United States Patent and Trademark Office. Questions related to size standards for a small business concern may be directed to Small Business Administration, Size Standards Staff, 409 Third Street, SW, Washington, DC 20416

I hereby state that rights under contract or law have been conveyed to and remain with the small business concern identified above with regard to the invention described in:

- ☒ the specification filed herewith with title as listed above.
☐ the application identified above.
☐ the patent identified above.

If the rights held by the above identified small business concern are not exclusive, each individual, concern, or organization having rights in the invention must file separate statements as to their status as small entities, and no rights to the invention are held by any person, other than the inventor, who would not qualify as an independent inventor under 37 CFR 1.9(c) if that person made the invention, or by any concern which would not qualify as a small business concern under 37 CFR 1.9(d), or a nonprofit organization under 37 CFR 1.9(e).

- Each person, concern, or organization having any rights in the invention is listed below:
☒ no such person, concern, or organization exists.
☐ each such person, concern, or organization is listed below.

Separate statements are required from each named person, concern or organization having rights to the invention stating their status as small entities. (37 CFR 1.27)

I acknowledge the duty to file, in this application or patent, notification of any change in status resulting in loss of entitlement to small entity status prior to paying, or at the time of paying, the earliest of the issue fee or any maintenance fee due after the date on which status as a small entity is no longer appropriate (37 CFR 1.28(b))

NAME OF PERSON SIGNING Michael Dougherty

TITLE OF PERSON IF OTHER THAN OWNER President and CEO

ADDRESS OF PERSON SIGNING 5110 Campus Drive, Plymouth Meeting, PA 19462

SIGNATURE  DATE 7/11/00

PROVISIONAL APPLICATION FOR UNITED STATES LETTERS PATENT

THERAPEUTIC USES FOR AMINOSTEROL COMPOUNDS

Inventors:

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Jon Williams
William Kinney
Mark Anderson
Mike McLane

THERAPEUTIC USES FOR AN AMINOSTEROL COMPOUND

RELATED APPLICATION DATA

5 This application is related to U.S. Patent Application 08/857,288 filed 5/16/97 which claims priority benefits under 35 U.S.C. § 119 based on U.S. Provisional Patent Appl. Nos. 60/017,627, filed May 17, 1996 and 60/029,541, filed November 1, 1996, which applications each are entirely incorporated herein by reference.

BACKGROUND OF THE INVENTION

I. INFORMATION RELATING TO PREVIOUS PATENTS AND APPLICATIONS

10 Several aminosterol compositions have been isolated from the liver and stomach of the dogfish shark, *Squalus acanthias*. One important aminosterol, squalamine, is the subject of U.S. Patent No. 5,192,756 to Zasloff, et al., which patent is entirely incorporated herein by reference. That patent describes the antibiotic properties of squalamine. Since the discovery of squalamine, several interesting properties of this compound have been discovered. For example, as described in U.S. Patent Nos. 5,733,899 and 5,721,226, squalamine may function as an antiangiogenic agent. These patents are entirely incorporated herein by reference. Additional uses of

20 squalamine (e.g., as an NHE3 inhibiting agent and as an agent for inhibiting the growth of endothelial cells) are disclosed in U.S. Patent No. 5,792,635 and U.S. Patent Appl. No. 08/840,706 (filed April 25, 1998, entitled "Treatment of Carcinomas Using Squalamine in Combination with Other Anti-cancer Agents," in the names of Michael Zasloff and Jon Williams) and continuation of this application, 09/150,724 filed 9/10/98 and PCT Application

25 US99/20645. These applications also are entirely incorporated herein by reference.

Methods for synthesizing squalamine have been devised, such as the methods described in WO 98/24800 (published June 10, 1998) and in U.S. Patent Appl. No. 60/032,378. This PCT publication and the U.S. patent application are entirely incorporated herein by reference. The PCT application relates to U.S. Patent Appl. No. 08/985,876, which application also is entirely incorporated herein by reference. Additionally, U.S. Patent No. 5,792,635 also discloses squalamine isolation and synthesis techniques.

Stemming from the discovery of squalamine, other aminosterols have been discovered in the dogfish shark liver and stomach and have been investigated. One important aminosterol that has been isolated and identified has the structure shown in Fig. 1. In this application, the compound having the structure shown in Fig. 1 will be referred to as "compound 1436" or simply "1436." This compound has the general molecular formula $C_{37}H_{72}N_4O_5S$ and a calculated molecular weight of 684.53017.

Compound 1436 previously has been described in U.S. Patent Nos. 5,795,885, 5,763,430, and 5,847,172. Each of these U.S. patents is entirely incorporated herein by reference. These U.S. patents describe the structure of compound 1436 and other aminosterols, as well as processes for synthesizing and isolating compound 1436 and related aminosterols. For example, compound 1436 may be prepared from squalamine as starting material. Additional methods for synthesizing compound 1436 (as well as squalamine) are described in U.S. Provisional Patent Appl. No. 60/032,378, filed December 6, 1996, which application is entirely incorporated herein by reference.

As further described in these patents and patent applications, compound 1436 has a variety of interesting properties. For example, compound 1436 has been found to be capable of

inhibiting mitogen-induced mouse, dog or human T-lymphocyte proliferation, as well as being capable of inhibiting the proliferation of a variety of other cells and tissues.

II. INFORMATION RELATING TO THIS APPLICATION

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Compound 1436 and other aminosterol compounds isolated from the dogfish shark liver and stomach have been found to possess interesting antibiotic and anti-proliferative properties with respect to a variety of cells and tissues. These interesting properties of compound 1436 have prompted applicants to conduct further investigation into the uses and properties of this compound.

10

In addition to treating various ailments and diseases, such as viral based ailments and diseases, cancers, and arthritis, compound 1436 has been found to have other favorable properties and effects. As one specific example, applicants have found that compound 1436 may be used to reduce weight gain in mammals and in a mouse model of type II diabetes to prevent weight gain and to maintain blood glucose at normal levels. The weight gain of the animals in these studies was controlled due to reduction of net food consumption and they were apparently healthy, viable animals.

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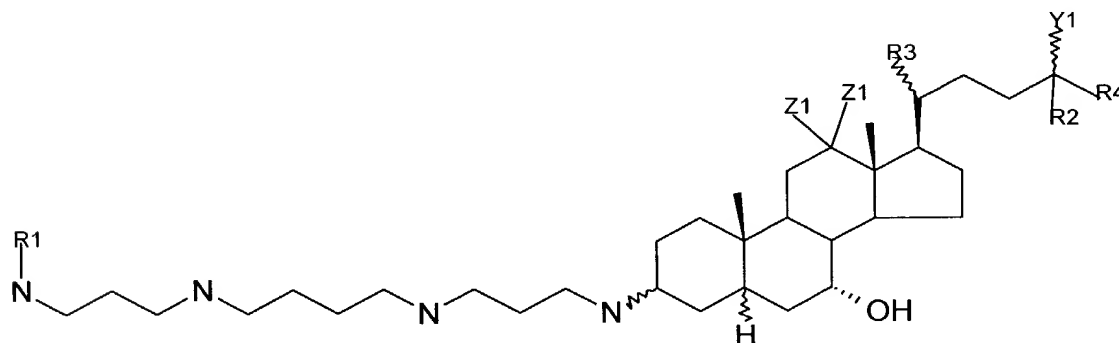
SUMMARY OF THE INVENTION

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This invention relates to a pharmaceutical composition, including a compound according to formula 1436 as shown in Fig. 1, or a pharmaceutically acceptable salt thereof (as an active ingredient), and a pharmaceutically acceptable carrier or excipient. The invention further relates to pharmaceutical products including the pharmaceutical composition described above. Such pharmaceutical products may be provided for the treatment of obesity and diabetes.

In another aspect of this invention it was determined that extended treatment of mice with the compound 1436 not only produced a inhibition of weight gain but also caused a reduction of serum cholesterol levels. Therefore one use for compound 1436 is for the reduction of serum cholesterol and the treatment of atherosclerosis.

5 In a further embodiment of the invention it was determined that analogues of the compound 1436 were also active in the prevention of weight gain in mice and it was found that the compounds were covered by the following generic formula:



R1=H or C1-C6 alkyl; R2=H or C1-C3 alkyl-X Where X=-H, -OH, -Cl, -Br, -I or -F;
R3=H or C1-C3 alkyl; R4=H or C1-C3 alkyl; Y1=-CO2H, -NH-SO2CF3, -SO3H,
-PO3H2, -OSO3H, -CF3 or -F; Z1=H or OH

BRIEF DESCRIPTION OF THE DRAWINGS

10 These and other advantageous aspects of the invention will be evident from the following detailed description, which should be considered in conjunction with the attached drawings, wherein:

Fig. 1 illustrates the molecular structure of aminosterol 1436;

15 Fig. 2 shows the effect of 1436 on mouse body weight in one study;

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Fig. 15 shows the effect of long term dosing of MSI-1436.

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To determine the long-term effects of MSI-1436 on the metabolic status of genetically obese mice, ob/ob animals were treated once weekly for 4 months (Figure 15). MSI-1436 produces a dose dependent reduction in weight gain. Animals receiving 10 or 20 mg/kg/wk lose

excessive weight and grow emaciated and moribund within several weeks (Figure 15).

However, safely managed weight control (i.e., no deaths) was possible over a four-month period using an intermittent dosing regimen (Figure 15 A, 3.1 mg/kg/week cohort). When weight fell to about 10% above starting weight, MSI-1436 dosing was stopped, permitting a continued but
5 limited decline in weight; as feeding increased, and weight recovered, dosing resumed. Ob/ob mice eventually develop diabetes, possibly because of fat accumulation within pancreatic islet cells (Chen G, Koyama K, Yuan X, Lee Y, Zhou Y-T, O'Doherty R, Newgard CB, Unger RH. Disappearance of body fat in normal rats induced by adenovirus-mediated leptin gene therapy. Proc. Natl. Acad. Sci. USA 1996; **93**: 14795-14799 and Shimabukuro M, Koyama K, Chen G, Wang M-Y, Trieu F, Lee Y, Newgard CB, Unger RH. Direct antidiabetic effect of leptin through triglyceride depletion of tissues. Proc. Natl. Acad. Sci. USA 1997; **94**: 4637-4641). Animals were evaluated about 40 days after treatment ceased (day 63). Mice receiving 3.1 mg/kg/week were within 10% above their starting weight, while untreated controls had gained 70%. An oral glucose tolerance test revealed a normal metabolic response in the MSI-1436 treated group, in contrast to the diabetic pattern in the vehicle-treated cohort (Figure 15 B). At 120 days, after dosing had resumed, serum cholesterol was normalized in animals with controlled weight gain (1.0 and 3.1 mg/kg/week) regardless of their absolute weight (Figure 15 C)

This invention further relates to various methods for using the pharmaceutical compositions in accordance with the invention. In the methods according to the invention,
20 various diseases or symptoms of diseases or ailments are treated by administering an effective amount of the above-described pharmaceutical composition. "Treat," "treated," or "treating," as used in this application may mean complete elimination of the disease, ailment, or symptoms, or it may mean reducing, suppressing, or ameliorating the severity of the disease, ailment, or

symptoms. As examples, certain body functions may be inhibited or enhanced by administering an effective amount of the above-described pharmaceutical compositions. In this manner, weight gain and growth factor production can be inhibited, or a diuretic effect can be produced by administering an effective amount of the pharmaceutical compositions in accordance with the invention. In a further embodiment of the invention compound 1436 can be used to treat type II diabetes where it can be used to prevent excessive weight gain and to control blood glucose levels.

This invention will be described below in terms of various specific examples and preferred embodiments. These examples and embodiments should be considered to be illustrative of the invention, and not as limiting the same.

EXAMPLE 1

BODY WEIGHT EFFECT OF COMPOUND 1436

Tests were conducted on compound 1436 to determine the effect of its administration on the body weight of mammals.

An animal model for testing the effectiveness of a compound against arthritis is the bovine type II Collagen Induced Arthritis Model in mice. Six to eight week old mice (male DBA/1LacJ from Jackson Labs) were separated into groups of 10. On Day 0, these mice were immunized subcutaneously with 100 μ g of bovine articular cartilage-derived type II collagen in an adjuvant system (RIBI Immunochem.). A booster injection of collagen in RIBI adjuvant was given on Day 21. Compound 1436 was dissolved in sterile, endotoxin-screened dH₂O and administered subcutaneously at 200 μ g/mouse (10 mg/kg). Dosing with 1436 was continued every fourth day (10 mg/kg) for the duration of the study. An additional group of immunized

mice (including 5 mice) was treated in this manner for the purpose of observing the weight-modulating effect of compound 1436. This additional group of 1436 treated mice, did not receive a collagen booster at Day 21. Like the other group of 1436 treated mice, these mice received their first 1436 dose on Day 17. The mice were weighed approximately weekly on the days indicated in Fig. 2.

Between Day 25 and Day 32, mice in the mouse serum albumin/PBS control group lost about 15% of their body weight, coinciding with the onset and progression of arthritis. See Fig. 2. A similar weight loss was observed in the 1436 treated mice, irrespective of whether the mice received the collagen booster injection at Day 21, and despite the fact that the 1436 treated mice were relatively disease free. While the body weight of the mouse serum albumen/PBS mice remained relatively constant after Day 32, the body weights of the 1436 treated mice (with and without the collagen booster) continued to decline through Day 39. Thus, this study indicates that 1436 may be useful to regulate weight gain. The Dex treated mice showed a stable weight pattern over the entire course of the study period.

In the Collagen Induced Arthritis Model described above, two of the five mice in the additional 1436 control group developed mild arthritis (clinical score of 2) despite having received no collagen booster immunization at Day 21. It was noted, however, that these mice received the priming collagen immunization at Day 0. This priming collagen injection is sufficient to induce arthritis in some animals.

Additional testing was performed to demonstrate the effect of compound 1436 on the body weight of mice. The dosage effect of compound 1436 was tested in one experiment. As illustrated in Fig. 3, compound 1436 was administered subcutaneously into BDF1 male mice in dosages of 1, 5 and 10 mg 1436/kg body weight every third day ("QTD") from Day 1 to Day 22.

The control vehicle and the 1436 administration vehicle in this experiment was 5% Dex in water. At 5 and 10 mg/kg 1436, weight gain was suppressed in these groups, and in fact, significant weight loss was experienced. Note the surprising decrease in the body weight of the mice treated with these doses of compound 1436. While these 1436 treated animals experienced a significant weight loss, however, they did not slow down and become lethargic. Rather, they were normally active and apparently healthy. 1 mg/kg 1436 did not appear to have a significant effect on the body weight of the mice in that group. Notably, after the 1436 treatment was stopped (at Day 22), weight gain returned in the animals treated with the 5 and 10 mg 1436/kg body weight doses. Eventually, after 1436 treatment was discontinued, the body weight of the animals in these groups reached the level of the animals in the other groups.

For oral dosing of compound 1436, as shown in Fig. 4, about 60 mg/kg active agent induced a weight suppression effect, which was most obvious on day 10 of the study. In this experiment, the CD-1 male mice were dosed orally with 60 mg/kg active once every third day (QTD) on days 0, 3 and 6, with a dosage volume of 0.01 ml/g. Note that 100 mg/kg 1436 is approximately equivalent to 60 mg/kg active. Again, after 1436 treatment was stopped, the body weight of the 1436 treated animals returned to the level of the animals in the control group.

In another experiment, genetically obese mice (OB/OB) and diabetic (db/db) mice were treated with compound 1436 to determine the effect on their body weight. Both homozygous (n=5) and heterozygous (n=5) control mice for both OB/OB and db/db mice were treated with sterile H₂O (vehicle). The test results are illustrated in Figs. 5 and 6. Weight loss and weight control were achieved for the 1436 treated mice. Figs. 5 and 6 show the effects of compound 1436 (administered s.c. at a dose of 10 mg/kg on days 0, 3 and 6 and 60) on weight in OB/OB

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and db/db mice, respectively. In Figs. 5 and 6, "OB/OB" and "db/db" relate to the leptin/leptin receptor.

The body weight suppression effect of compound 1436 may be the result of suppression of growth factor production and/or growth factor release in the treated animals. To test this possibility, the effect of compound 1436 on release of growth hormone during a GHRF stimulatory test was studied. Compound 1436 or saline (vehicle) was administered at 10 mg/kg i.v. to rats 30 minutes prior to a growth hormone releasing factor (GHRF) challenge (2.5 µg GHRF/kg, i.v. at t=0), and the plasma growth hormone output was measured. Blood samples were taken at t=0, 5, 10 and 15 minutes. As illustrated in Fig. 7, compound 1436 was found to inhibit growth hormone secretion in rats during a GHRF provocation test. In this test, the GHRF is added in this system to induce an increase in growth hormone release.

To determine the roles of food and water consumption as well as urine output of compound 1436 on body weight suppression, 20 DBA/2J mice were randomly assigned to one of four groups (5 mice/group) that received either vehicle or compound 1436 at 1, 2.5, or 5 mg active/kg (i.p. every third day ("q 3d") for 4 dosings (i.e., dosed on Days 0, 3, 6 and 9)). Using metabolic cages for accurate measurements, group food consumption, group water consumption, and group urine output were measured daily, and individual body weight were monitored three times per week. Figure 8 shows a dose-dependent change in body weight. Figure 9 shows that food consumption of the group receiving compound 1436 at 5 mg/kg was lower than the other groups from Day 3 through Day 7 and lower than vehicle or low dose 1436 from Day 7 through 13. The mid-dose 1436 group had lower food consumption than control or low dose on Days 7 through 14. These reductions in food consumption preceded body weight loss by a few days; the lag would be consistent with a depletion of endogenous fuel storage (fat, glycogen, etc.). Figures

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10 and 11 show a dose-dependent increase in urine output and water consumption that temporarily aligns with reductions in body weight. Water balance in the body is composed of intake via ingestion and formation of metabolic water and output is via urine, feces and insensible loss (perspiration, respiration). This study only measured water ingested and urine
5 output so the animals may be in a negative water balance, even though water consumption was in excess of urine output. This study suggests that both food consumption as well as a diuresis (negative water balance) may contribute to the suppression of body weight.

In a separate arm of the study, 10 CD-1 mice (5 mice/group) were randomly assigned to receive either compound 1436 or vehicle as control administered intraperitoneally once per day, every day ("QD") for 13 days (Days 0 to 12). Body weights were monitored daily, and blood samples were obtained on Day 13 (one day post-last administration). Plasma levels of electrolytes (in mEq) and osmolality (in mOsm) were measured. The results in Fig. 12 show that compound 1436 caused weight loss without changing the isotonicity or electrolyte levels of plasma compared to vehicle control mice.

EXAMPLE 2

EFFECT OF COMPOUND 1436 ON SERUM GLUCOSE IN DB/DB MICE

Age matched animals (4 females/group) were treated with MSI-1436, 10mg/kg via the i.p. route every 3 days for a total of 4 doses. Animals were provided food and water *ad lib*. At day 0, db/db (untreated), db/db (treated), and db/+ (untreated) cohorts weighed 28.1 ± 2.2 ,
20 30.8 ± 1.3 and 17.8 ± 0.9 gms, respectively. At day 20, 7 hours after the last feeding, blood was drawn and glucose determined using a standard clinical glucose meter. Animals were sacrificed and the body composition of the carcasses was determined by published methods (Pellymounter, M.A., M.J. Cullen, M.B. Baker, R. Hecht, D. Winters, T. Boone, and F. Collins. 1995. Effects of

the *obese* gene product on body weight regulation in ob/ob mice. Science **269**: 540-543). Over the 20 day period of the study, the weight of untreated homozygotes increased from about 28 to 39 grams. Treated animals dropped from about 31 to 20 grams, losing significant fat and water stores as well as a small but significant fraction of lean mass (Table 1). Treated homozygotes retained more fat than a comparable group of phenotypically normal(but untreated) db/+ heterozygotes (Table 1). Note that the severe hyperglycemia of the db/db homozygote was corrected, reduced from 465.0 mg/dl to 53.5 mg/dl (Table 1).

TABLE 1

Effects of MSI-1436 on body composition and serum glucose in obese mice

Mice	Treatment	Body Composition				Serum		
		Water		Fat		Lean mass		Glucose(mg/dl)
		g	%	g	%	g	%	
db/db	saline	12.6	32.4	20.7	52.8	5.8	14.8	465.0
db/db	MSI-1436	7.3 ^b	36.6	8.7 ^c	42.8 ^a	4.2 ^a	20.6 ^c	53.5 ^c
db/+	saline	10.4	53.6	3.9	19.6	4.9	25.5	94.8

P-values (two-sided) are based on comparison of the treated and untreated db/db groups. **a**, 0.01 < p ≤ 0.05; **b**, 0.001 < p ≤ 0.01; **c**, p ≤ 0.001

EXAMPLE 3

EFFECT OF MSI-1436 AND STRUCTURAL ANALOGUES ON BODY WEIGHT OF CD-1

MICE

Experiments were performed using age-matched (4-5 weeks old) male mice (Jackson Laboratories) provided food and water *ad lib*. Mice corresponding to a single experimental

cohort were housed together in a single cage. Animals were used 1 week after arrival. Lights were on from 0600 to 1800 and room temperature was kept at $21 \pm 2^\circ\text{C}$. Daily at 0800-1000 animals were weighed, food and water replaced. Drug dosing was conducted with 10mg/kg of each compound via the i.p. route every 3 days for 4 doses, beginning on day 0. The structures of the compounds used is shown in Figure 13 and the results in Figure 14. Analogues that differ from MSI-1436 at only a single stereocenter, such as MSI-1701 (the polyamine at C-3) or MSI-1777 (the methyl at C-20) exhibit significantly reduced activity, while MSI-1673 (the OH at C-7) is completely inactive. The naturally occurring aminosterol, squalamine, identical to MSI-1436 except for a difference in the polyamine, is inactive highlighting the importance of a precise polyamine structure (not shown). This can be further demonstrated by the analogue MSI-1750, identical to MSI-1436, but differing by the substitution of the two central amino groups of the spermine moiety with oxygens. Additions of longer alkyl groups on the primary amine of the MSI-1436 molecule also reduces activity (MSI-1888), as does the addition of a CH_2OH group on C-24 (MSI-1839). The presence of the sulfate on the C-24 hydroxyl moiety is required for activity (MSI-1521) (not shown). However, chirality of the C-24 hydroxyl itself appears to be relatively unimportant (MSI-1814). These studies suggest that MSI-1436 affects weight loss through a specific pathway, rather than as a consequence of a "non-specific" side effect of this chemical class of substance.

EXAMPLE 4

EXTENDED DOSING OF OB/OB MICE WITH MSI-1436

Male ob/ob mice received weekly dosing of MSI-1436. All doses were administered via the i.p. route (8 animals/ dosing level) on Mondays in the A.M. Animals were provided standard

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mouse chow and water *ad lib*. Dosing was stopped (Figure 15 A, large circles) or resumed (Figure 15 A, large X) at various times, as noted. Animals in the 20mg/kg and 10mg/kg groups were euthanized at days 16 and 30, respectively due to extreme emaciation. Animals being treated weekly at 3.1 mg/kg/week stopped receiving MSI-1436 at day 21. MSI-1436 produces a dose dependent reduction in weight gain. Animals receiving 10 or 20 mg/kg/wk lose excessive weight and grow emaciated and moribund within several weeks (Figure 15 A). However, safely managed weight control (i.e., no deaths) was possible over a four-month period using an intermittent dosing regimen (Figure 15 A, 3.1 mg/kg/week cohort). When weight fell to about 10% above starting weight, MSI-1436 dosing was stopped, permitting a continued but limited decline in weight; as feeding increased, and weight recovered, dosing resumed.

Following overnight restriction of food, an oral glucose tolerance test was conducted on day 65 on this group, on an untreated group of ob/ob mice, and on a cohort of wt/wt mice of the ob/ob background strain (C57BL/6J) of the same age. Each animal received 5 ml/kg of 30 % glucose by gavage. Blood was drawn by tail vein puncture at indicated times and blood glucose measured used a clinical glucose-meter (Life Scan, model One-touch II) (Figure 15 B). The test revealed a normal metabolic response in the MSI-1436 treated group, in contrast to the diabetic pattern in the vehicle-treated cohort (Figure 15 B).

Serum cholesterol was measured at study termination. Animals had received their last doses of MSI-1436 one week prior. Blood was drawn after overnight food restriction, and cholesterol measured by automated methodology (Vet-Test model 8008, IDEXX Labs) (Figure 15 C). It was seen that serum cholesterol was normalized in animals with controlled weight gain (1.0 and 3.1 mg/kg/week) regardless of their absolute weight (Figure 15 C).

III. THERAPEUTIC ADMINISTRATION AND COMPOSITIONS

The mode of administration of compound 1436 and the other aminosterol compounds may be selected to suit the particular therapeutic use. Also, the compound can be administered to any subject for whom treatment is believed to be beneficial, but administration to humans or other mammals is particularly preferred in the invention. Modes of administration generally include, but are not limited to, transdermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, inhalation, intralesional, endothelial, and oral routes. The compounds may be administered by any convenient route, for example, by infusion or bolus injection, or by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal, and intestinal mucosa, etc.), and the active 1436 ingredient may be administered together with other biologically active agents. Administration may be local or systemic.

In this application, the abbreviation "s.q." or "s.c." is used to represent subcutaneous administration of compound 1436 or other substances. The abbreviation "i.p." is used to represent intraperitoneal administration of compound 1436 or other substances. The abbreviation "i.v." is used to represent intravenous administration of compound 1436 or other substances. The abbreviation "i.m." is used to represent intramuscular administration of compound 1436 or other substances. In certain figures attached to this application, one graph axis is labeled "RT." This stands for "reverse transcriptase," which relates to the manner in which a viral enzyme can copy a RNA molecule into a DNA copy. Those skilled in the art are familiar with this measurement technique.

The present invention also provides pharmaceutical compositions that include compound 1436 or another aminosterol compound as an active ingredient. Such pharmaceutical compositions include a therapeutically effective amount of compound 1436 (or a

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pharmaceutically acceptable salt thereof) or another aminosterol compound (or a pharmaceutically acceptable salt thereof) and a pharmaceutically acceptable carrier or excipient. Examples of such a carrier include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol, and combinations thereof. The particular form and formulation of the pharmaceutical composition should be selected to suit the mode of administration and can be determined and selected by the skilled artisan, e.g., through routine experimentation.

The pharmaceutical composition, if desired, also may contain minor amounts of other conventional agents, such as wetting or emulsifying agents, or pH buffering agents. The pharmaceutical composition may be in any suitable form, such as a liquid solution, suspension, emulsion, tablet, pill, capsule, sustained release formulation, or powder. The pharmaceutical composition also may be formulated as a suppository, with traditional binders and carriers, such as triglycerides. Oral formulations may include standard carriers, such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc.

Various delivery systems are known and may be used to administer a therapeutic compound of the invention, e.g., encapsulation in liposomes, microparticles, microcapsules and the like.

In one embodiment, the pharmaceutical composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to humans. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the pharmaceutical composition also may include a solubilizing agent and a local anesthetic to ameliorate pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example,

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as a dry lyophilized powder or water-free concentrate in a hermetically sealed container such as an ampule or sachet indicating the quantity of active agent. Where the pharmaceutical composition is to be administered by infusion, it may be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the pharmaceutical composition is administered by injection, an ampoule of sterile water for injection or saline may be provided so that the ingredients may be mixed prior to administration.

The amount of the therapeutic compound of the invention that will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and this amount can be determined by standard clinical techniques known to those skilled in the art through routine experimentation. The precise dose to be employed in the pharmaceutical composition also will depend on the route of administration and the seriousness of the disease or disorder, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective therapeutical doses may be determined from extrapolations of dose-response curves derived from *in vitro* or animal-model test systems.

The following dosage ranges are exemplary. Suitable dosages for intravenous administration are generally about 20 micrograms to 40 milligrams of active compound per kilogram body weight. Suitable dosage ranges for intranasal administration are generally about 0.01 mg/kg body weight to 1 mg/kg body weight. Suitable dosage ranges for topical administration are generally at least about 0.01% by weight. Suitable dosages for oral administration are generally about 500 micrograms to 800 milligrams per kilogram body weight, and preferably about 1-200 mg/kg body weight. Suppositories generally contain, as the active ingredient, 0.5 to 10% by weight of the aminosterol active ingredient. Oral formulations preferably contain 10% to 95% active ingredient.

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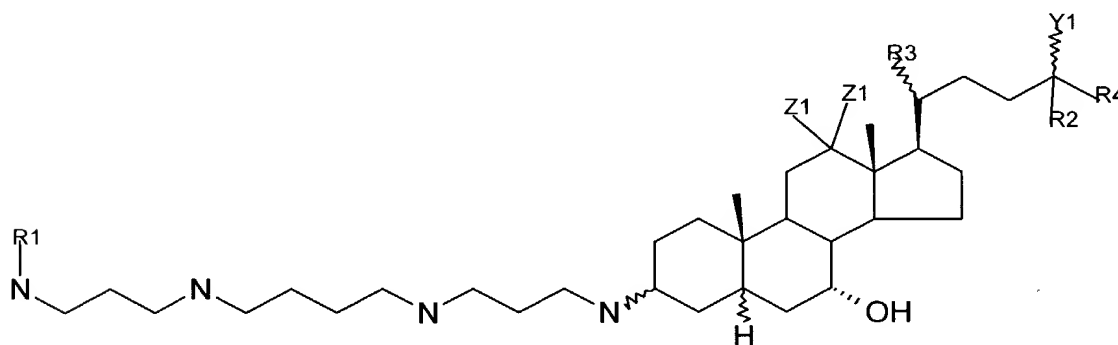
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In describing the invention, applicant has stated certain theories in an effort to disclose how and why the invention works in the manner in which it works. These theories are set forth for informational purposes only. Applicants do not wish to be bound by any specific theory of operation.

WE CLAIM:

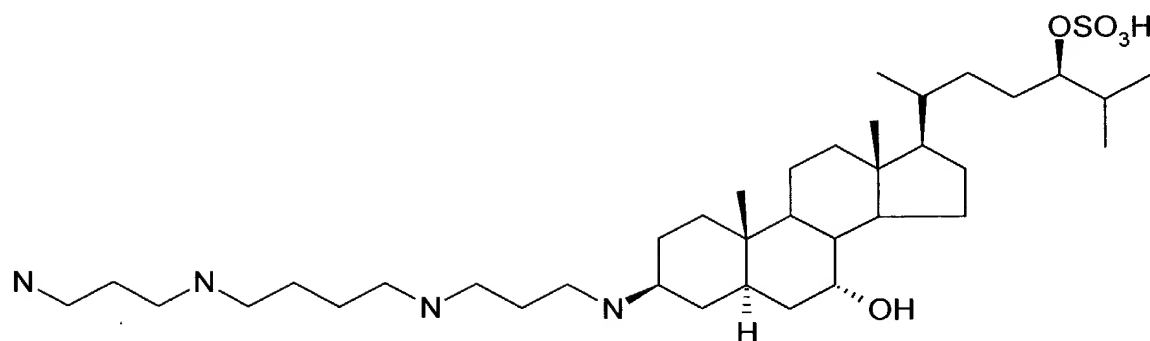
1. A method for treating a disease or ailment selected from the group consisting of:
reduction of serum cholesterol, atherosclerosis, obesity and diabetes, the method comprising:
administering an effective amount of a composition, wherein the composition includes a
pharmaceutically acceptable carrier or excipient, and a compound according to the following
formula:



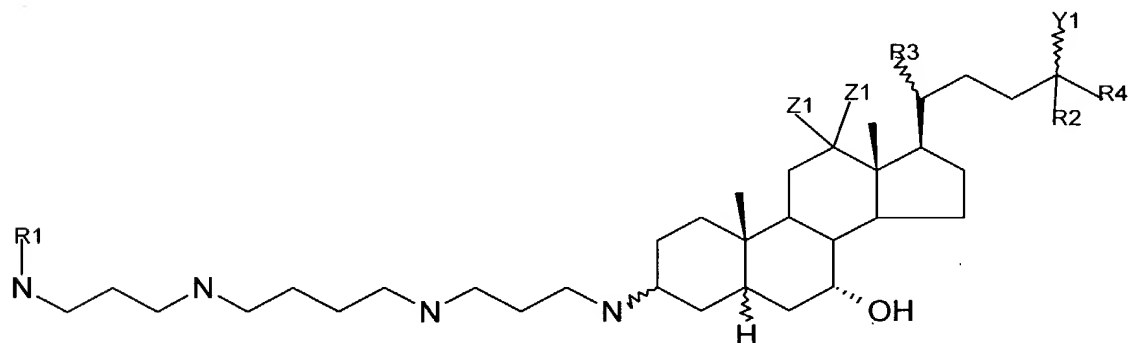
R1=H or C1-C6 alkyl; R2=H or C1-C3 alkyl-X Where X=-H, -OH, -Cl, -Br, -I or -F;
R3=H or C1-C3 alkyl; R4=H or C1-C3 alkyl; Y1=-CO₂H, -NH-SO₂CF₃, -SO₃H,
-PO₃H₂, -OSO₃H, -CF₃ or -F; Z1=H or OH

or a pharmaceutically acceptable salt thereof.

2. A method according to claim 1, wherein the disease or ailment is diabetes.
3. A method according to claim 1, wherein the disease or ailment is obesity.
4. A method according to claim 1, wherein the disease or ailment is atherosclerosis.
5. A method according to claim 1, wherein the disease or ailment is elevated serum cholesterol.
6. A method according to claim 1, wherein the compound has the following formula:



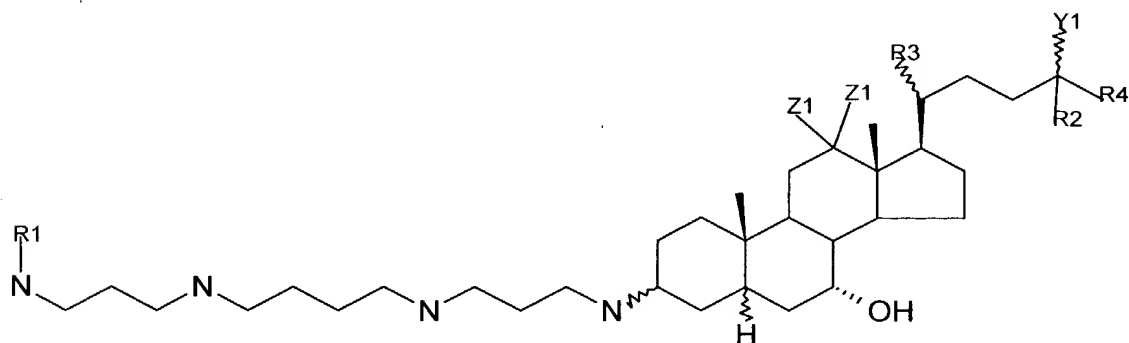
7. A method for suppressing appetite in a mammal, comprising: administering an effective amount of a composition, wherein the composition includes a pharmaceutically acceptable carrier or excipient, and a compound according to the following formula:



R1=H or C1-C6 alkyl; R2=H or C1-C3 alkyl-X Where X=-H, -OH, -Cl, -Br, -I or -F;
 R3=H or C1-C3 alkyl; R4=H or C1-C3 alkyl; Y1=-CO₂H, -NH-SO₂CF₃, -SO₃H,
 -PO₃H₂, -OSO₃H, -CF₃ or -F; Z1=H or OH

or a pharmaceutically acceptable salt thereof.

8. A method for inhibiting growth factor production in a mammal, comprising:
administering an effective amount of a composition, wherein the composition includes a
pharmaceutically acceptable carrier or excipient, and a compound according to the following
formula:



R1=H or C1-C6 alkyl; R2=H or C1-C3 alkyl-X Where X=-H, -OH, -Cl, -Br, -I or -F;
R3=H or C1-C3 alkyl; R4=H or C1-C3 alkyl; Y1=-CO2H, -NH-SO2CF3, -SO3H,
-PO3H2, -OSO3H, -CF3 or -F; Z1=H or OH

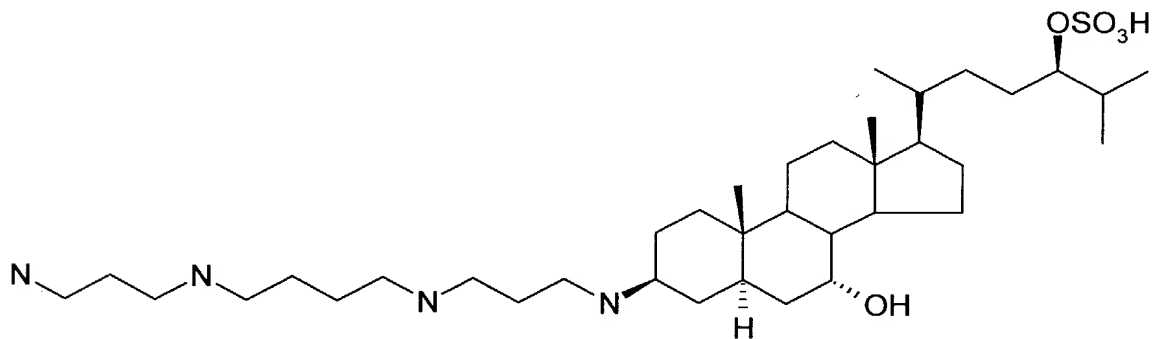
or a pharmaceutically acceptable salt thereof.

9. A method for inhibiting weight gain in a mammal, comprising: administering an
effective amount of a composition, wherein the composition includes a pharmaceutically

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CC(C)[C@H](OS(=O)(=O)O)CC[C@H](C)[C@H]1CC[C@@H]2[C@@]1(CC[C@H]3[C@H]2CC=C4[C@@]3(CC[C@@H](C4)O)C)C

10. A method for reducing blood glucose levels in a mammal, comprising: administering an effective amount of a composition, wherein the composition includes a pharmaceutically acceptable carrier or excipient, and a compound according to the following formula:



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H
E
L
P
S
O
F
T
W
A
R
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11. A method according to claim 7, wherein the control of the blood glucose level is useful for the treatment of diabetes.

CC(C)[C@H](OS(=O)(=O)O)CC[C@H](C)[C@H]1CC[C@@H]2[C@@]1(CC[C@H]3[C@H]2CC=C4[C@@]3(CC[C@@H](C4)O)C[C@H]5[C@@H]([C@@H](C2)NCCCN)CCCN)CCN

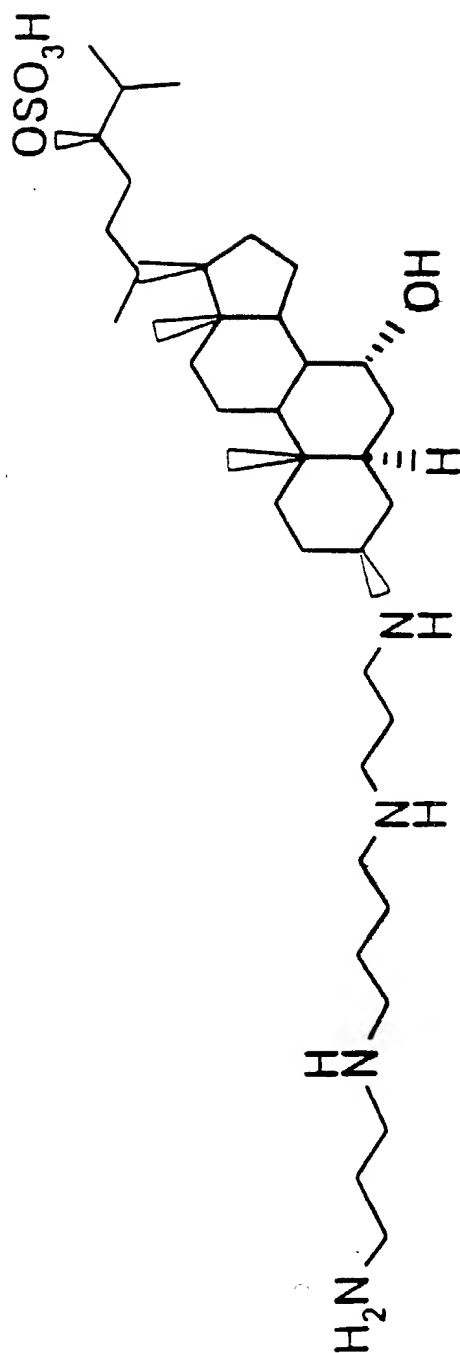
13. A method according to claim 12, wherein the control of the blood cholesterol level is useful for the treatment of atherosclerosis.

ABSTRACT OF THE DISCLOSURE

A pharmaceutical composition includes, as an active ingredient, a compound according to formula 1436 as shown in Fig. 1, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier or excipient. Various pharmaceutical products may be produced including this pharmaceutical composition. Such pharmaceutical products may be used for the treatment of obesity or diabetes. Methods for using the pharmaceutical compositions also are described. In these methods, various diseases are treated or other body functions are activated or inhibited by administering an effective amount of the pharmaceutical composition. For example, diabetes and obesity may be treated by administering an effective amount of the pharmaceutical compositions. Weight gain, and growth factor production can be inhibited by administering an effective amount of these pharmaceutical compositions. Appetite can be suppressed by administering an effective amount of the pharmaceutical compositions, and a diuretic effect can be produced.

FIG. 1

COMPOUND-1436



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FIGURE 2

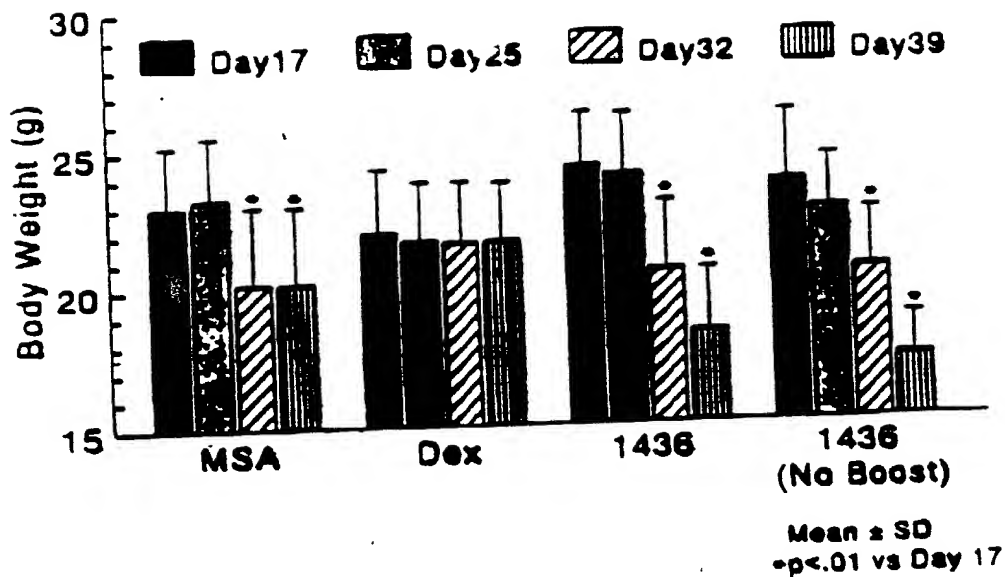
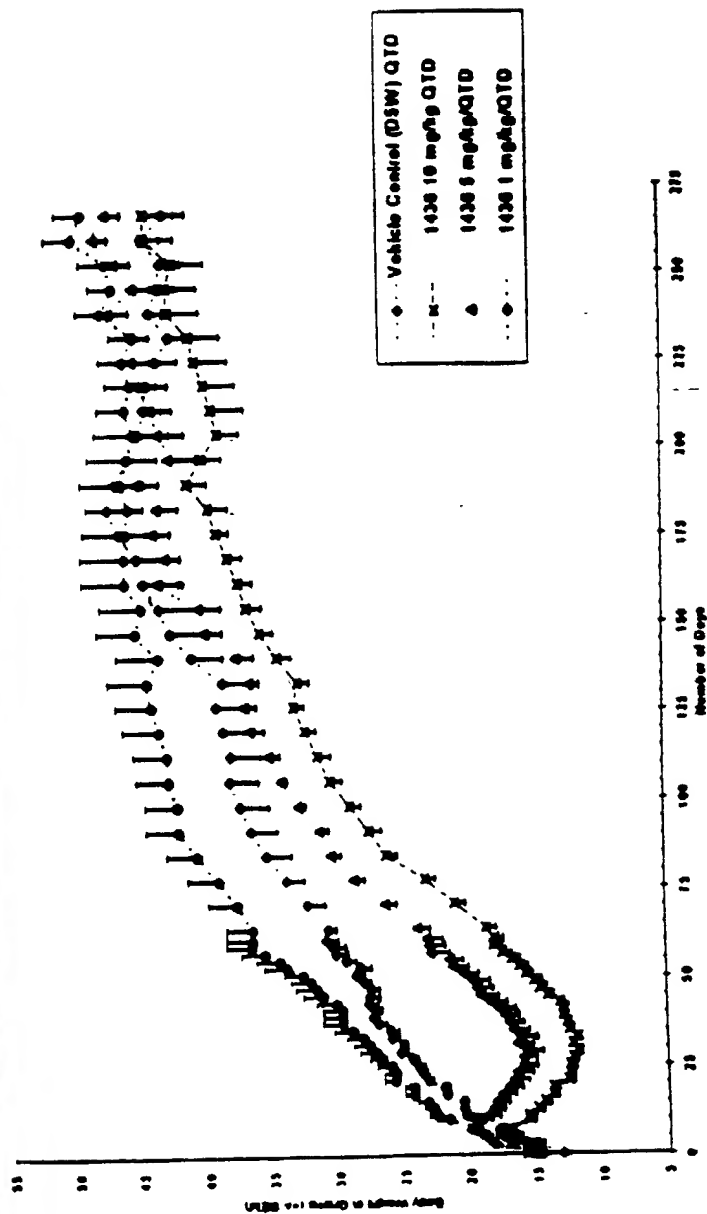


FIGURE 3

Effect of Compound-1436 On The Body Weight of BDF-1 Male Mice



Dosage volume 0.01 cc/g. First day of treatment Day 1. Last day of treatment day 22.

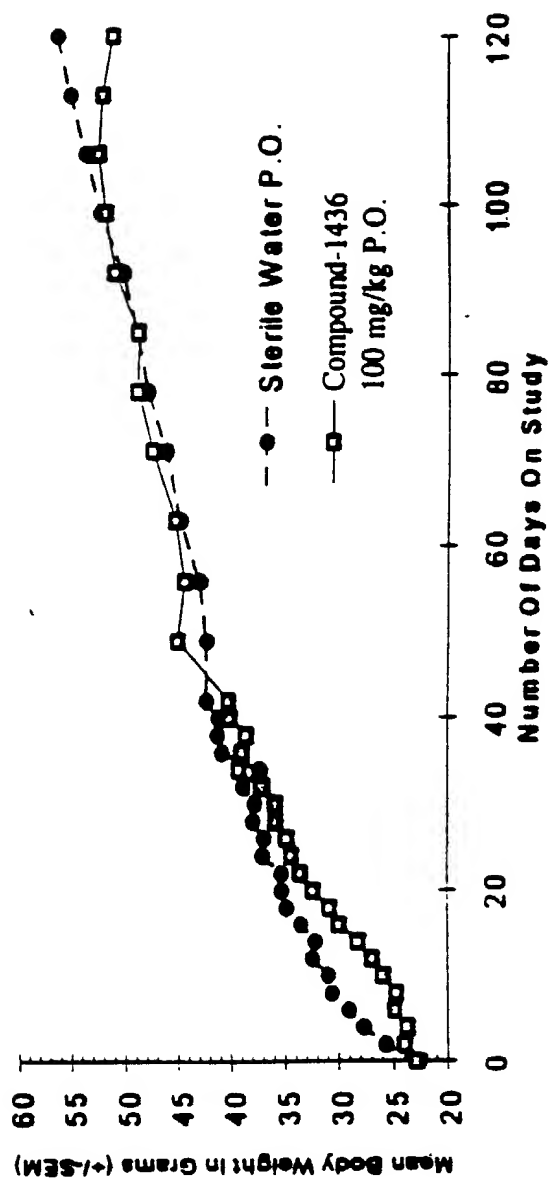
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FIGURE 4

Effects of Oral Dosing of Compound-1436 On The Body Weights of Male CD-1 Mice



All mice dosed QTD. Dosage Volume 0.01 mL/g. First day of treatment is day 0. Last day of Treatment is Day 6.

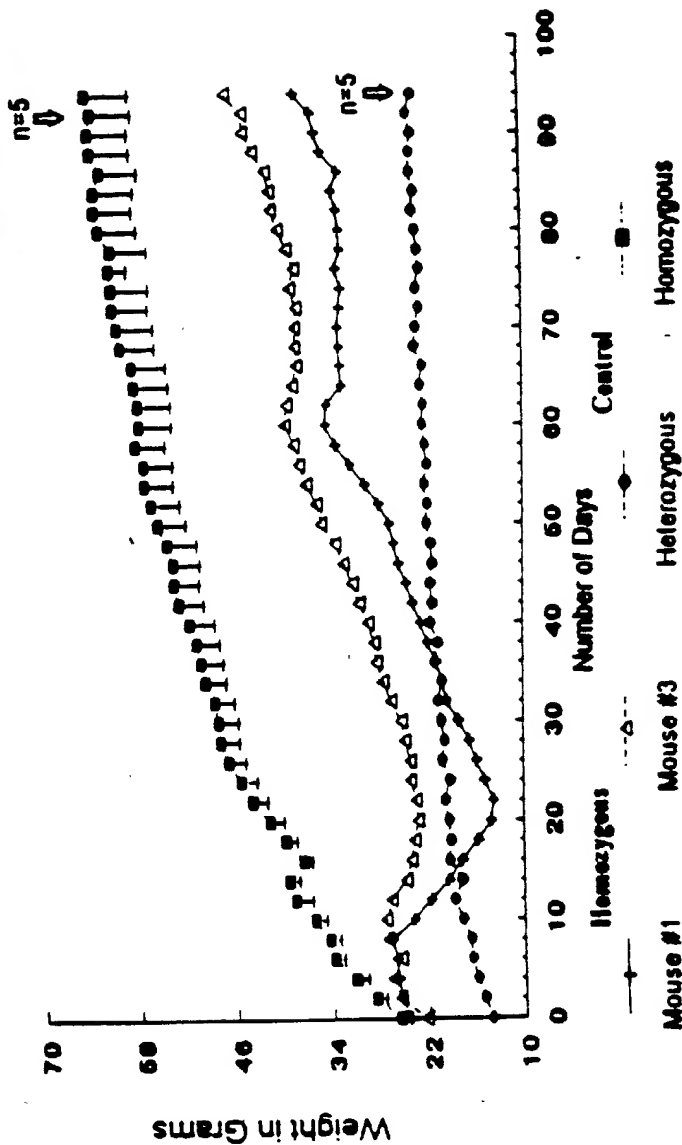
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FIGURE 5

Effect of Compound-1436 on Weight in OB/OB Mice



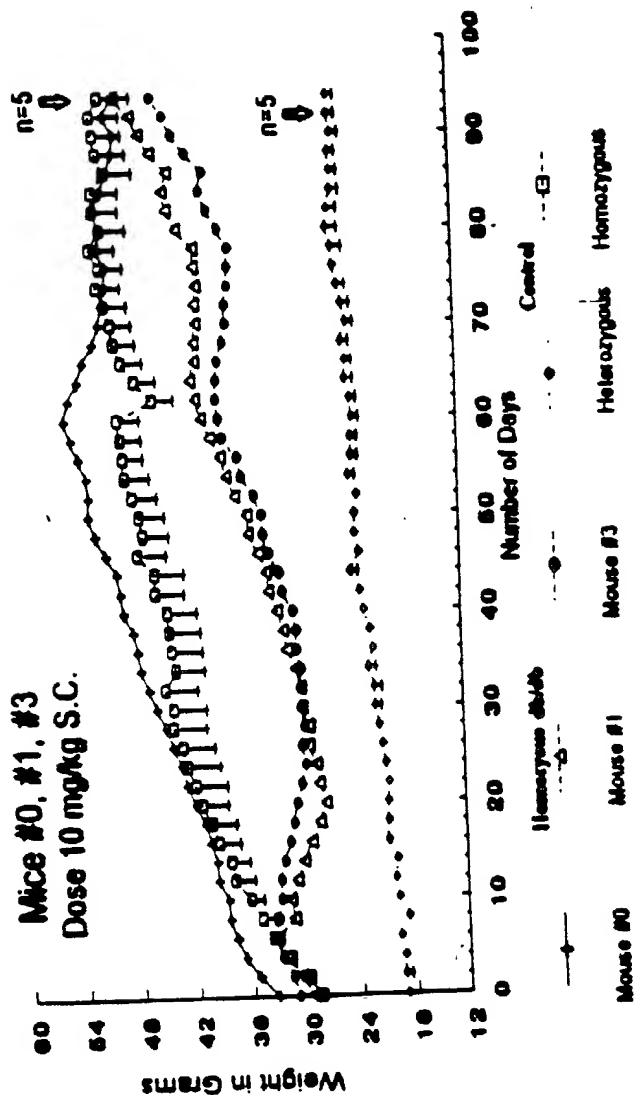
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FIGURE 6

Effect of Compound-1436 on Weight in db/db Mice



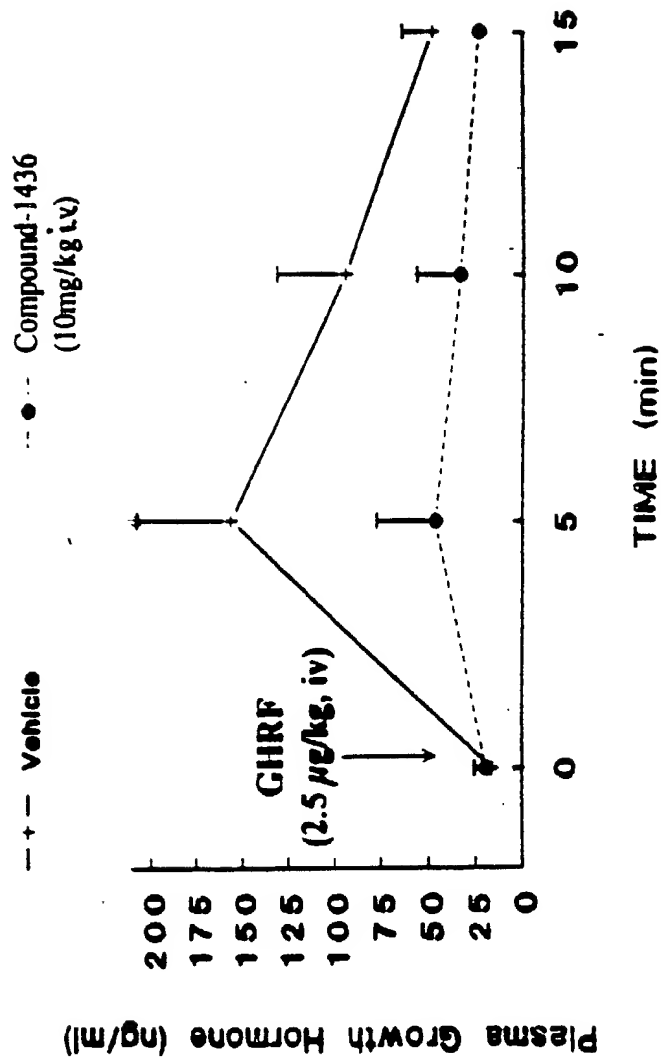
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FIGURE 7

Compound-1436 Inhibits GHRF-Induced Increase in Rat GH Levels In Vivo



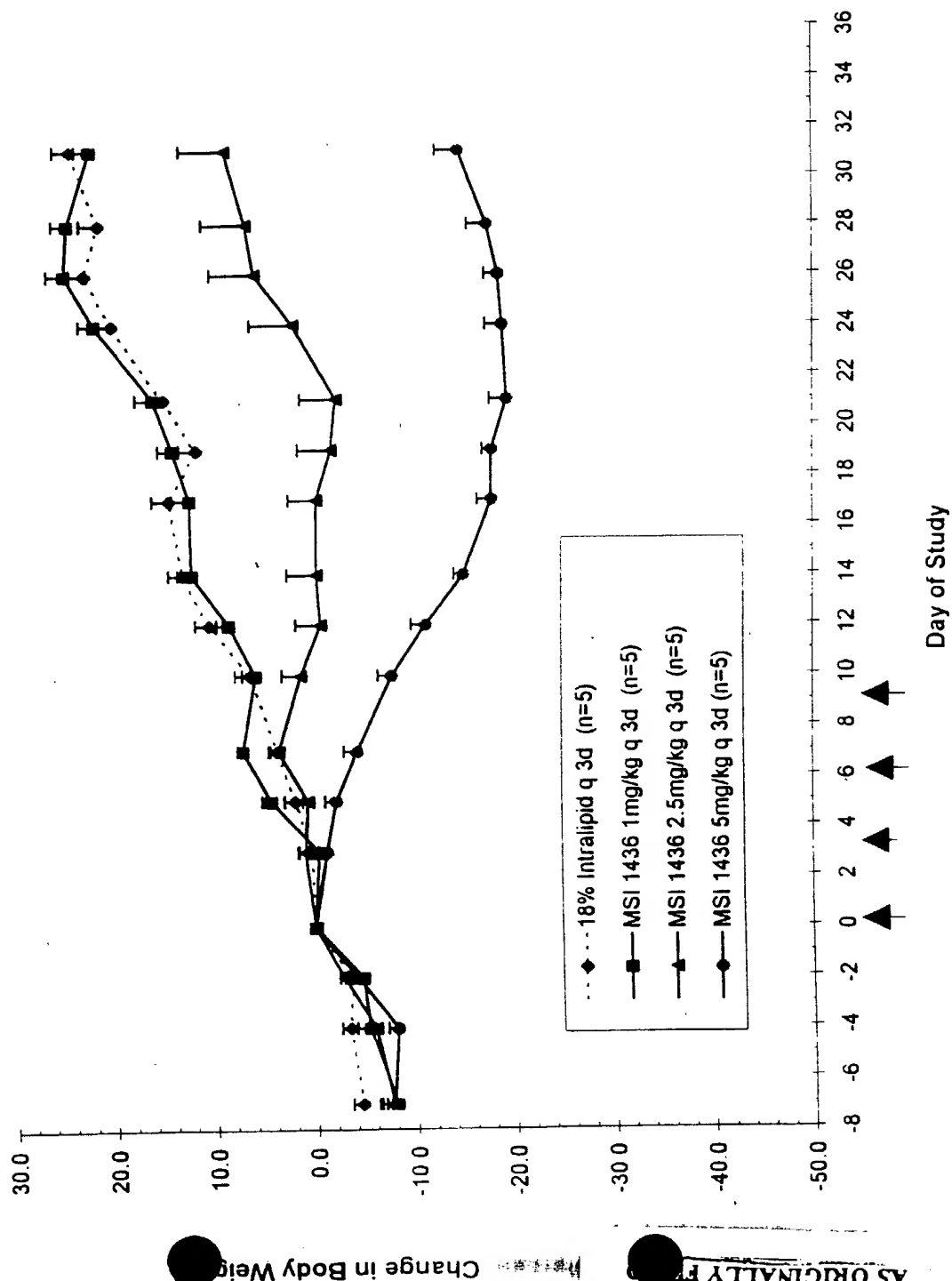
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FIGURE 8

The Effect of MSI-1436 (1, 2.5, or 5mg/kg IP q 3d) on Body Weight in DBA/2J Mice



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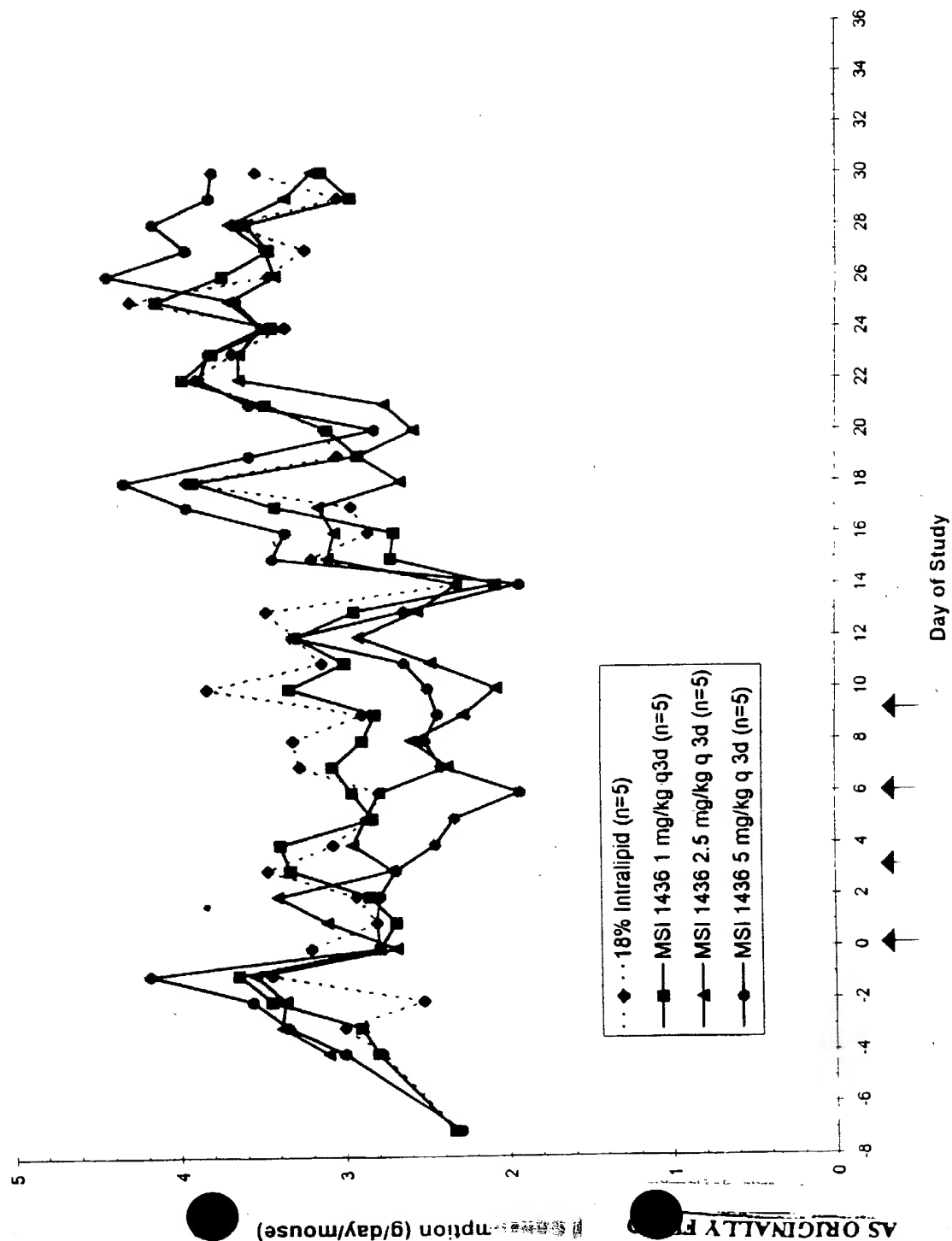
Change in Body Weight

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FIGURE 9

The Effect of MSI 1436 (1, 2.5, or 5mg/kg q3d) on Food Consumption of DBA/2J Mice



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FIGURE 11

Effect of MSI 1436 (1, 2.5, or 5 mg/kg q 3d) on Water Consumption in DBA/2J Mice

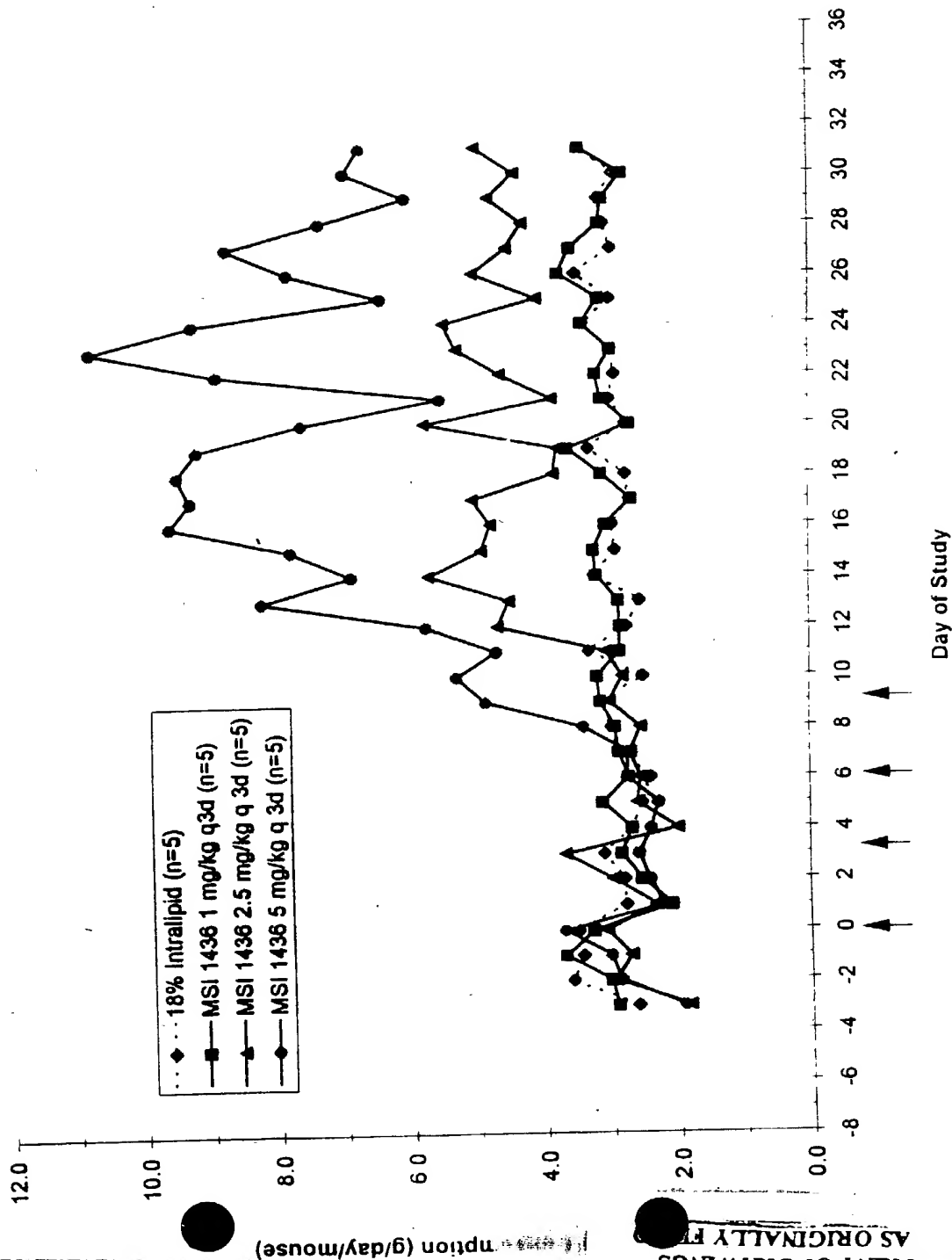
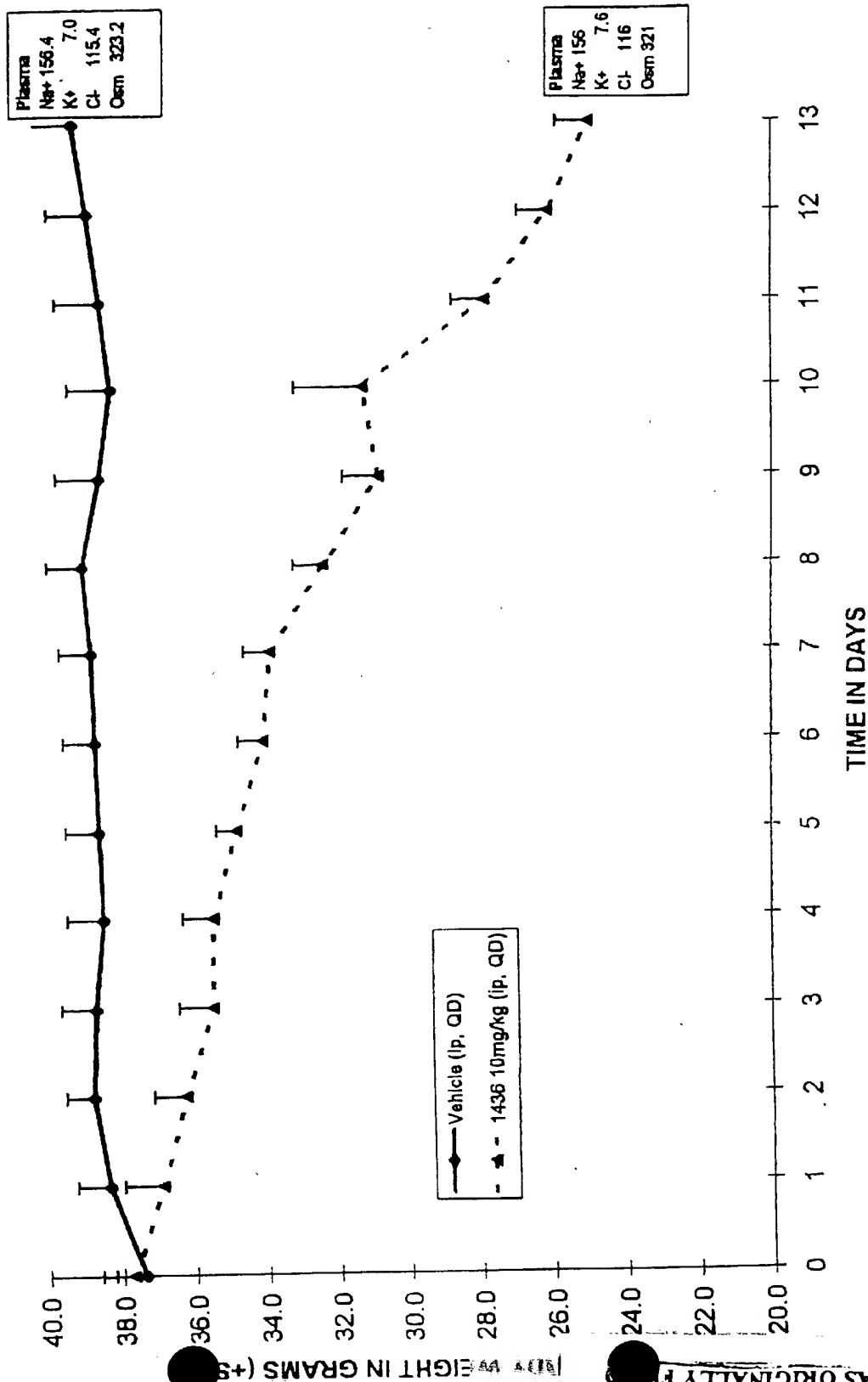


FIGURE 12

EFFECTS OF MSI 1436 ON BODY WEIGHT AND PLASMA ELECTROLYTES IN CD-1 MICE



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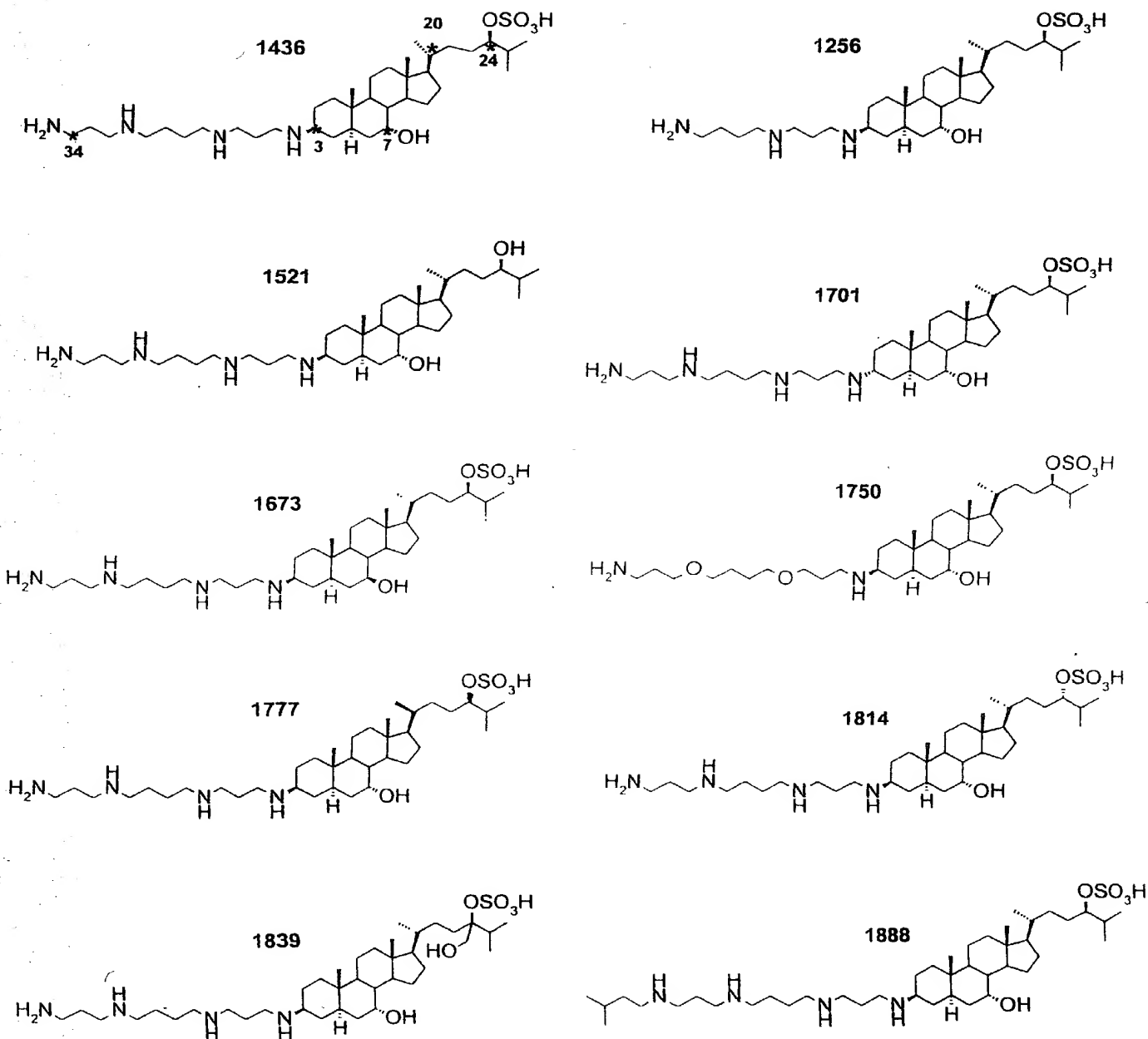


Fig. 13

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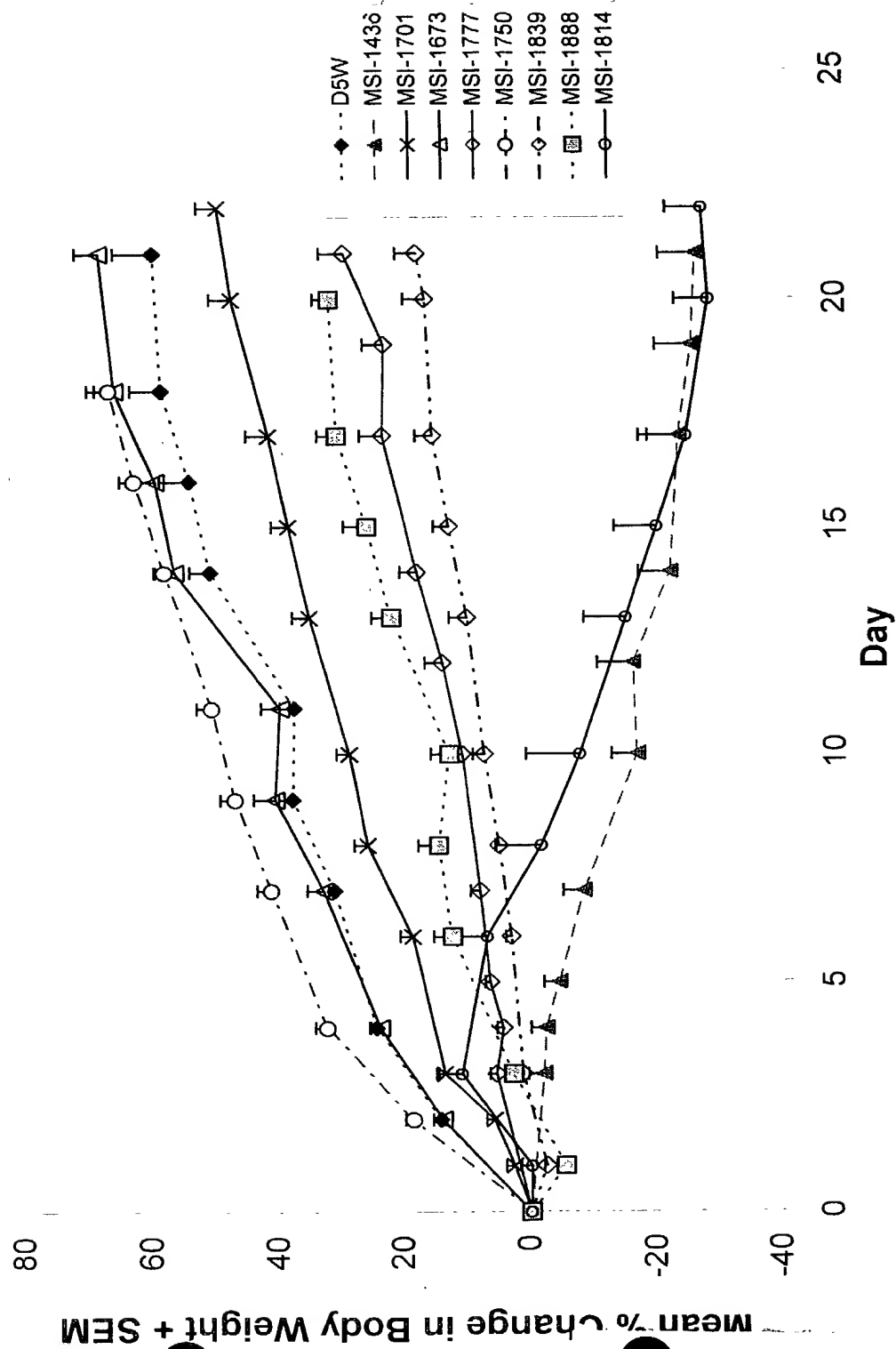


Fig. 14
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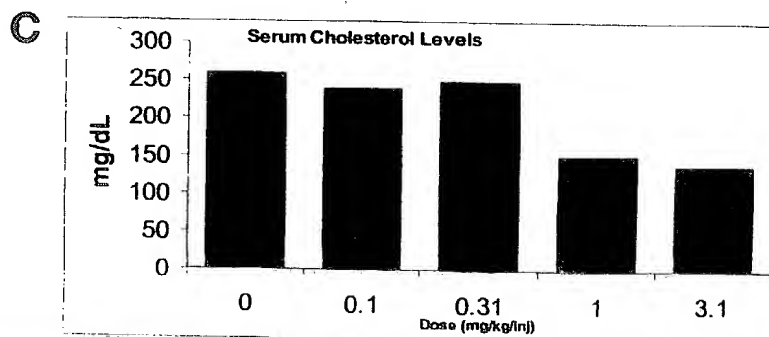
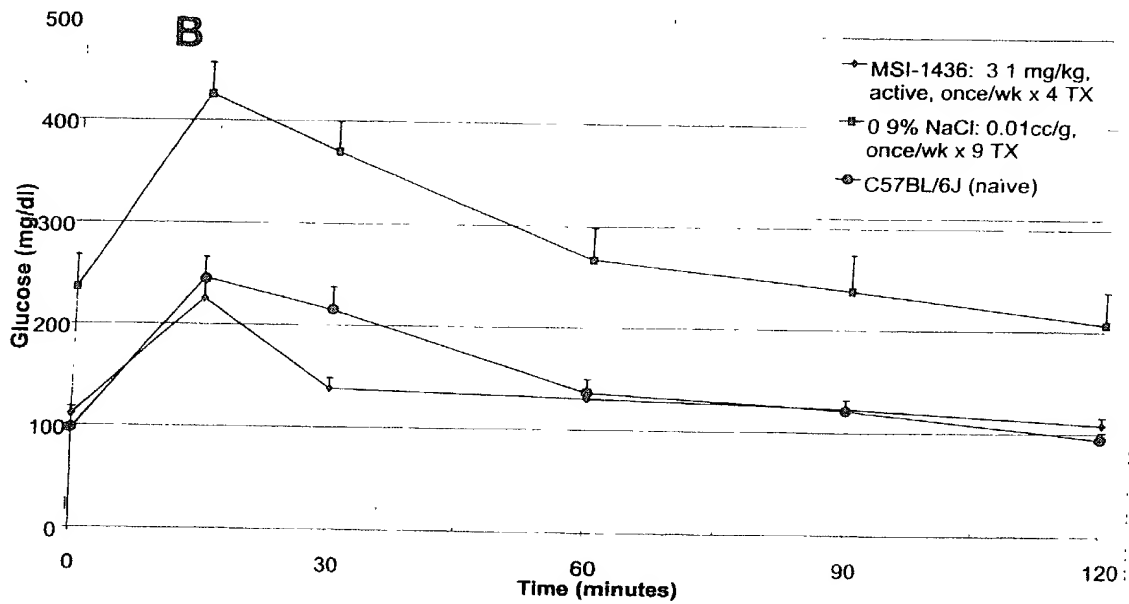
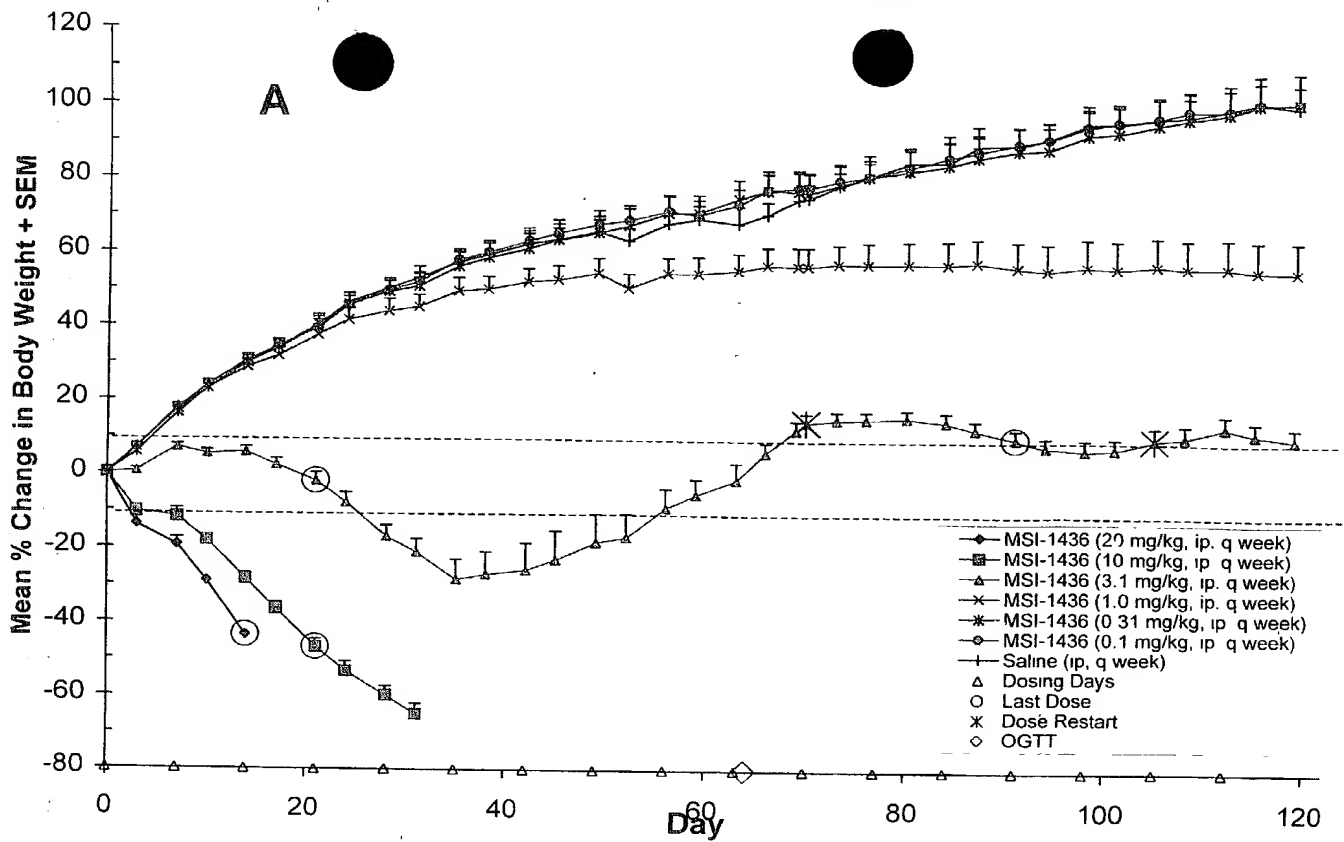


Fig-15